APPENDIX 4

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1. (Amended) A method for obtaining a bioactivity or a biomolecule of interest, comprising:

a) screening a library of clones generated from nucleic acids obtained directly from a mixed population of cells, for a specified bioactivity or biomolecule;

b) mutating a nucleic acid sequence contained in a clone from the library having the specified bioactivity or biomolecule; and

c) comparing the bioactivity or biomolecule from b) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of sequence mutation, thereby providing the bioactivity or biomolecule of interest.

2. The method of claim 1, wherein the biomolecule is a nucleic acid sequence.

3. The method of claim 2, wherein the nucleic acid sequence is a DNA or RNA sequence.

4. The method of claim 2, wherein the nucleic acid sequence is screened by contacting the nucleic acids contained in the clone with at least one oligonucleotide probe comprising a detectable molecule and at least a portion of the nucleic acid sequence of interest; and identifying nucleic acid sequences containing a complement to the at least one oligonucleotide probe with an analyzer that detects a detectable signal from the detectable molecule.

5. The method of claim 4, wherein the detectable molecule is a chromogenic or a fluorogenic substrate.

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6. (Amended) The method of claim 4, wherein the detectable signal is fluorescence.

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7. (Amended) The method of claim 5, wherein the fluorogenic substrate is umbelliferone or a derivative thereof, resorufin or a derivative thereof, fluorescein or a derivative thereof, or rhodamine or a derivative thereof.

8. The method of claim 4, wherein the detectable molecule is a detectably labeled oligonucleotide having a sequence encoding a polypeptide of interest or a fragment thereof.

9. The method of claim 8, wherein the detectably labeled oligonucleotide is labeled with a fluorescent molecule.

10. The method of claim 2, wherein the screening is by PCR amplification of a nucleic acid sequence of interest using primers substantially complementary to the sequence of interest or sequences flanking a nucleic acid of interest and having a detectable molecule.

11. The method of claim 2, wherein the screening is by hybridization of an oligonucleotide substantially complementary to a nucleic acid sequence of interest and having a detectable molecule.

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12. (Amended) The method of claim 2, further comprising comparing the mutated nucleic acid sequence of interest to the non-mutated nucleic acid sequence to identify the nucleotide sequence mutation.

13. The method of claim 12, wherein the comparison is performed using a sequence comparison algorithm.

14. The method of claim 1, wherein the bioactivity is provided by a polypeptide.

15. The method of claim 1, wherein the bioactivity is an enzymatic activity.

16. The method of claim 15, wherein the enzymatic activity is provided by an enzyme selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases, lignin peroxidases, diarylpropane peroxidases, epozide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.

17. The method of claim 1, wherein the library is an expression library.

18. The method of claim 1, wherein the library contains DNA obtained from an environmental sample.

19. The method of claim 1, wherein the library contains DNA obtained from extremophiles.

20. The method of claim 19, wherein the extremophiles are thermophiles.

21. The method of claim 20, wherein the extremeophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles.

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22. (Amended) The method of claim 17, wherein the screening comprises contacting a clone with a substrate wherein interaction of the substrate with the bioactivity or biomolecule contained in the clone produces a detectable signal.

24. The method of claim 22, wherein the bioactive substrate comprises C12FDG.

25. The method of claim 22, wherein the substrate comprises a first test protein linked to a DNA binding moiety and a second test protein linked to a transcriptional activation moiety, wherein modulation of the interaction of the first test protein linked to the DNA binding moiety with the second test protein linked to the transcription activation moiety results in a change in the expression of a detectable protein.

26. The method of claim 22, wherein the screening is by expression of nucleic acid.

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27. (Amended) The method of claim 1, further comprising, prior to (a), obtaining nucleic acids from the clone containing the specified bioactivity or biomolecule.

28. The method of claim 27, wherein obtaining the nucleic acids contained in the clone comprises contacting the clone with a complementary nucleic acid, or fragment thereof, thereby allowing hybridization of the clone nucleic acids with the complementary nucleic acid and isolation thereof.

29. The method of claim 28, wherein the complementary nucleic acid or fragment thereof comprises a solid phase bound hybridization probe.

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30. (Amended) The method of claim 1, wherein the nucleic acid sequence is mutated by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, ligation reassembly, GSSM and any combination thereof.

31. (Amended) The method of claim 1, wherein nucleic acid sequence is mutated by error-prone PCR.

32. (Amended) The method of claim 1, wherein the nucleic acid sequence is mutated by shuffling.

33. (Amended) The method of claim 1, wherein the nucleic acid sequence is mutated by oligonucleotide-directed mutagenesis.

34. (Amended) The method of claim 1, wherein the nucleic acid sequence is mutated by assembly PCR.

35. (Amended) The method of claim 1, wherein the nucleic acid sequence is mutated by sexual PCR mutagenesis.

36. (Amended) The method of claim 1, wherein the nucleic acid sequence is mutated by *in vivo* mutagenesis.

37. (Amended) The method of claim 1, wherein the nucleic acid sequence is mutated by cassette mutagenesis.

38. (Amended) The method of claim 1, wherein the nucleic acid sequence is mutated by recursive ensemble mutagenesis.

39. (Amended) The method of claim 1, wherein the nucleic acid sequence is mutated by exponential ensemble mutagenesis.

40. (Amended) The method of claim 1, wherein the nucleic acid sequence is mutated by site-specific mutagenesis.

41. (Amended) The method of claim 1, comprising screening the clone of (b) for a further specified protein or enzymatic activity prior to mutating the nucleic acids.

42. The method of claim 1, wherein the library is generated in a prokaryotic cell.

43. The method of claim 1, wherein the library is generated in a *Streptomyces sp.*

44. The method of claim 43, wherein the Streptomyces *is Streptomyces venezuelae.*

45. The method of claim 42, wherein the prokaryotic cell is gram negative.

46. The method of claim 42, wherein the prokaryotic cell is a *Bacillus sp.*

47. The method of claim 42, wherein the prokaryotic cell is a *Pseudomonas sp.*

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48. (Amended) The method of claim 1, wherein the library is screened by contacting a clone of the library with a substrate, wherein a bioactivity or biomolecule produced by the clone is detectable by a difference in the substrate prior to contacting with the clone as compared to after contacting.

49. The method of claim 1, wherein the library is normalized before screening the library.

50. The method of claim 1, wherein the bioactivity or biomolecule is a gene cluster or fragment thereof.

51. The method of claim 1, wherein the bioactivity or biomolecule is a polypeptide in a metabolic pathway.

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52. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) screening a library of clones generated from pooled nucleic acids obtained directly from a plurality of isolates for a specified bioactivity or biomolecule; and

b) identifying a clone which contains the specified bioactivity or biomolecule.

53. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) screening a library of clones generated from pooled nucleic acids obtained directly from a plurality of isolates for a specified bioactivity or biomolecule;

b) mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

c) comparing the bioactivity or biomolecule from b) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence mutation, thereby providing the bioactivity or biomolecule of interest.

54. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) screening a library of clones generated from pooling individual gene libraries generated from the nucleic acids obtained directly from each of a plurality of isolates for a specified bioactivity or biomolecule; and

b) identifying a clone which contains the specified bioactivity or biomolecule.

55. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) screening a library for a specified bioactivity or biomolecule wherein the library is generated from pooling individual gene libraries generated from the nucleic acids obtained directly from each of a plurality of isolates;

b) mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

c) comparing the bioactivity or biomolecule from c) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence mutation, thereby providing the bioactivity or biomolecule of interest.

56. A method of identifying a bioactivity or biomolecule of interest, comprising:

(a) screening a library of clones generated from the nucleic acids from an enriched population of organisms for a specified bioactivity or biomolecule; and

(b) identifying a clone containing the specified bioactivity or biomolecule.

57. (Amended) A method of identifying a bioactivity or biomolecule of interest, comprising:

a) screening a library of clones generated from nucleic acids obtained directly from an enriched population of organisms for a specified bioactivity or biomolecule;

b) mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

c) comparing the bioactivity or biomolecule from b) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence mutation, thereby providing the bioactivity or biomolecule of interest.

58. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

(a) incubating nucleic acids obtained directly from a mixed population of organisms with at least one oligonucleotide probe comprising a detectable molecule and at least a portion of a nucleic acid sequence encoding a molecule of interest under such conditions and such time to allow interaction of complementary sequences;

(b) identifying nucleic acid sequences having a complement to the oligonucleotide probe using an analyzer that detects the detectable molecule;

(c) generating a library from the identified nucleic acid sequences;

(d) screening the library for a specified bioactivity or biomolecule;

(e) mutating a nucleic acid sequence contained in a clone from the library having the specified bioactivity or biomolecule; and

(f) comparing the bioactivity or biomolecule product from (e) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence mutation, thereby providing the bioactivity or biomolecule of interest

59. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

(a) co-encapsulating in a microenvironment nucleic acids obtained directly from a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable molecule and at least a portion of a nucleic acid sequence encoding a molecule of interest under such conditions and for such time as to allow interaction of complementary sequences;

(b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable molecule;

(c) generating a library from the separated encapsulated nucleic acids;

(d) screening the library for a specified bioactivity or biomolecule;

(e) mutating a nucleic acid sequence contained in a clone from the library having the specified bioactivity or biomolecule; and

(f) comparing the bioactivity or biomolecule product from (e) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence mutation, thereby providing the bioactivity or biomolecule of interest.

60. (Amended) A method for- identifying a bioactivity or a biomolecule of interest, comprising:

(a) co-encapsulating in a microenvironment nucleic acids obtained directly from an isolate of a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;

(b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule or interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;

(c) gencrating a library from the separated encapsulated nucleic acids;

(d) screening the library for a specified bioactivity or biomolecule;

(e) mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

(f) comparing the bioactivity or biomolecule product from (e) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence mutation, thereby providing the bioactivity or biomolecule of interest.

61. (Amended) A method for obtaining a bioactivity or a biomolecule of interest, comprising:

(a) co-encapsulating in a microenvironment nucleic acids obtained directly from one or more isolates of a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;

(b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;

(c) generating a library from the separated encapsulated nucleic acids;

(d) screening the library for a specified bioactivity or biomoleeule;

(e) mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

(f) comparing the bioactivity or biomolecule product from (e) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence mutation, thereby providing the bioactivity or biomolecule of interest.

62. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) co-encapsulating in a microenvironment nucleic acids obtained directly from a mixture of isolates of a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;

b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;

c) generating a library from the separated encapsulated nucleic acids;

d) screening the library for a specified bioactivity or biomolecule;

e) mutating the a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

f) comparing the bioactivity or biomolecule product from (e) with the specified bioactivity or biomolecule wherein a difference in the bioactivity or biomolecule is indicative of an effect of introducing at least one sequence mutation, thereby providing the bioactivity or biomolecule of interest.

63. (Amended) A method for obtaining a bioactivity or a biomolecule of interest, comprising:

a) screening a library of clones generated from nucleic acids obtained directly from a mixed population of cells, for a specified bioactivity or biomolecule;

b) mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

c) screening the mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

64. The method of claim 63, wherein the biomolecule is a nucleic acid sequence.

65. The method of claim 64, wherein the nucleic acid sequence is a DNA or RNA sequence.

66. The method of claim 64, wherein the nucleic acid sequence is screened by contacting the nucleic acids contained in the clone with at least one oligonucleotide probe comprising a detectable molecule and at least a portion of the nucleic acid sequence of interest; and identifying nucleic acid sequences containing a complement to the at least one oligonucleotide probe with an analyzer that detects a detectable signal from the detectable molecule.

67. The method of claim 66, wherein the detectable molecule is a chromogenic or a fluorogenic substrate.

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68. (Amended) The method of claim 66, wherein the detectable signal is fluorescence.

69. (Amended) The method of claim 67, wherein the fluorogenic substrate is umbelliferone or a derivative thereof, resorufin or a derivative thereof, fluorescein or a derivative thereof, or rhodamine or a derivative thereof.

70. The method of claim 66, wherein the detectable molecule is a detectably labeled oligonucleotide having a sequence encoding a polypeptide of interest or a fragment thereof.

71. The method of claim 70, wherein the detectably labeled oligonucleotide is labeled with a fluorescent molecule.

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72. (Amended) The method of claim 63, wherein the screening is by PCR amplification of a nucleic acid sequence of interest using primers substantially complementary to the sequence of interest or sequences flanking a nucleic acid of interest and having a detectable molecule.

73. (Amended) The method of claim 63, wherein the screening is by hybridization of an oligonucleotide substantially complementary to a nucleic acid sequence of interest and having a detectable molecule.

74. The method of claim 63, wherein the bioactivity is provided by a polypeptide.

75. The method of claim 63, wherein the bioactivity is an enzymatic activity.

76. The method of claim 75, wherein the enzymatic activity is provided by an enzyme selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, mono- and dioxygenases, haloperoxidases, lignin

peroxidases, diarylpropane peroxidases, epozide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.

77. The method of claim 63, wherein the library is an expression library.

78. The method of claim 63, wherein the library contains DNA obtained from an environmental sample.

79. The method of claim 78, wherein the environmental sample is selected from ice, water, permafrost, material of volcanic origin, soil and plants.

80. The method of claim 63, wherein the library contains DNA obtained from extremophiles.

81. The method of claim 80, wherein the extremophiles are thermophiles.

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82. (Amended) The method of claim 80, wherein the extremeophiles are selected from the group consisting of hyperthermophiles, psychrophiles, halophiles, psychrotrophs, alkalophiles, and acidophiles.

83. The method of claim 63, wherein the screening comprises contacting a clone with a substrate labeled with a detectable molecule wherein interaction of the substrate with the bioactivity or biomolecule contained in the clone produces a detectable signal.

84. The method of claim 83, wherein the substrate is a bioactive substrate.

85. The method of claim 83, wherein the bioactive substrate comprises C12FDG.

86. The method of claim 83, wherein the screening is by expression of nucleic acid.

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87. (Amended) The method of claim 63, wherein the nucleic acid sequence is mutated by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential

Cont C 15

ensemble mutagenesis, site-specific mutagenesis, ligation reassembly, GSSM and any combination thereof.

88. (Amended) The method of claim 63, wherein the nucleic acid sequence is mutated by error-prone PCR.

89. (Amended) The method of claim 63, wherein the nucleic acid sequence is mutated by shuffling.

90. (Amended) The method of claim 63, wherein the nucleic acid sequence is mutated by oligonucleotide-directed mutagenesis.

91. (Amended) The method of claim 63, wherein the nucleic acid sequence is mutated by assembly PCR.

92. (Amended) The method of claim 63, wherein the nucleic acid sequence is mutated by sexual PCR mutagenesis.

93. (Amended) The method of claim 63, wherein the nucleic acid sequence is mutated by *in vivo* mutagenesis.

94. (Amended) The method of claim 63, wherein the nucleic acid sequence is mutated by cassette mutagenesis.

95. (Amended) The method of claim 63, wherein the nucleic acid sequence is mutated by recursive ensemble mutagenesis.

96. (Amended) The method of claim 63, wherein the nucleic acid sequence is mutated by exponential ensemble mutagenesis.

97. (Amended) The method of claim 63, wherein the nucleic acid sequence is mutated by site-specific mutagenesis.

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98. (Amended) The method of claim 63, comprising screening the clone of (c) for a further specified protein or enzymatic activity, prior to mutating the nucleic acids.

99. The method of claim 63, wherein the library is generated in a prokaryotic cell.

100. The method of claim 63, wherein the library is generated in a *Streptomyces sp.*

101. The method of claim 100, wherein the Streptomyces is *Streptomyces venezuelae.*

102. The method of claim 99, wherein the prokaryotic cell is gram negative.

103. The method of claim 99, wherein the prokaryotic cell is a *Bacillus sp.*

104. The method of claim 99, wherein the prokaryotic cell is a *Pseudomonas sp.*

C16

105. (Amended) The method of claim 63, wherein the library is screened by contacting a clone of the library with a substrate, wherein a bioactivity or biomolecule produced by the clone is detectable by a difference in the substrate prior to contacting with the clone as compared to after contacting.

106. The method of claim 63, wherein the library is normalized before screening the library.

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107. (Amended) The method of claim 63, wherein the biomolecule is a gene cluster or fragment thereof.

108. (Amended) The method of claim 63, wherein the biomolecule is a polypeptide in a metabolic pathway.

109. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) screening a library for a specified bioactivity or biomolecule wherein the library is generated from pooling individual gene libraries generated from the nucleic acids obtained directly from each of a plurality of isolates;

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b) mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

c) screening the mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

110. (Amended) A method of identifying a bioactivity or biomolecule of interest, comprising:

a) screening a library of clones generated from nucleic acids obtained directly from an enriched population of organisms for a specified bioactivity or biomolecule;

b) mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

c) screening the mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

111. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) incubating nucleic acids obtained directly from a mixed population of organisms with at least one oligonucleotide probe comprising a detectable molecule and at least a portion of a nucleic acid sequence encoding a molecule of interest under such conditions and such time to allow interaction of complementary sequences;

b) identifying nucleic acid sequences having a complement to the oligonucleotide probe using an analyzer that detects the detectable molecule;

c) generating a library from the identified nucleic acid sequences;

d) screening the library for a specified bioactivity or biomolecule;

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e) mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

f) screening the mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

112. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) co-encapsulating in a microenvironment nucleic acids obtained directly from a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable molecule and at least a portion of a nucleic acid sequence encoding a molecule of interest under such conditions and for such time as to allow interaction of complementary sequences;

b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable molecule;

c) generating a library from the separated encapsulated nucleic acids;

d) screening the library for a specified bioactivity or biomolecule;

e) mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

f) screening the mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

113. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) co-encapsulating in a microenvironment nucleic acids obtained directly from an isolate of a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;

b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;

c) generating a library from the separated encapsulated nucleic acids;

d) screening the library for a specified bioactivity or biomolecule;

e) mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

f) screening the mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

114. (Amended) A method for obtaining a bioactivity or a biomolecule of interest, comprising:

a) co-encapsulating in a microenvironment nucleic acids obtained directly from one or more isolates of a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;

b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;

c) generating a library from the separated encapsulated nucleic acids;

d) screening the library for a specified bioactivity or biomolecule;

e) mutating a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

f) screening the mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

115. (Amended) A method for identifying a bioactivity or a biomolecule of interest, comprising:

a) co-encapsulating in a microenvironment nucleic acids obtained directly from a mixture of isolates of a mixed population of organisms, with at least one oligonucleotide probe comprising a detectable marker and at least a portion of a polynucleotide sequence encoding a molecule having a bioactivity of interest under such conditions and for such time as to allow interaction of complementary sequences;

b) identifying encapsulated nucleic acids containing a complement to the oligonucleotide probe encoding the molecule of interest by separating the encapsulated nucleic acids with an analyzer that detects the detectable marker;

c) generating a library from the separated encapsulated nucleic acids;

d) screening the library for a specified bioactivity or biomolecule;

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e) mutating the a nucleic acid sequence contained in a clone having the specified bioactivity or biomolecule; and

f) screening the mutated nucleic acid sequence to obtain the biomolecule or the bioactivity of interest.

116. A method for obtaining a desired bioactivity or biomolecule, comprising: creating a DNA library comprised of DNA molecules obtained directly from an environmental source; introducing at least one mutation into a DNA molecule from said library to create a mutagenized DNA molecule; and screening for a desired bioactivity or biomolecule containing a mutation.

117. The method of claim 116, wherein the DNA molecules are obtained from uncultivated organisms in the environmental source.

118. The method of claim 117, wherein introduction of the at least one mutation comprises directed evolution mutagenesis.

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119. (Amended) The method of claim 116, further comprising the step of: expressing the mutagenized molecule to create a bioactivity or biomolecule containing a mutation.

120. The method of claim 116, wherein the DNA molecules are genomic DNA.

132. The method of claim 116, wherein the mutagenized DNA molecule includes a gene cluster.

133. The method of claim 116, wherein the mutagenized DNA comprises one or more operons, or portions thereof.

134. The method of claim 133, wherein the operon, or portions thereof, encodes a complete or partial metabolic pathway.

C19 135. (Amended) The method of claim 133, wherein the operon produces a polyketide synthase.

136. The method of claim 116, wherein the molecule is suitable for veterinary use.

C20 137. (Amended) The method of claim 116, wherein the DNA molecules are inserted into a vector prior to said creating a DNA library.

138. The method of claim 137, wherein the vector is selected from viral particles, baculovirus, phage, plasmids, phagemids, cosmids, fosmids, bacterial artificial chromosomes, and viral DNA.

139. The method of claim 116, wherein the environmental sample is selected from, soil, water, permafrost, materials of volcanic origin, and plants.

140. The method of claim 139, wherein the environmental sample is obtained from Arctic, Antarctic or tropical areas.

141. The method of claim 116, wherein the DNA molecules obtained in step a) are enriching for a particular organism or organisms of interest.

142. The method of claim 116, where the DNA molecules are derived from a plurality of donor organisms.

143. The method of claim 116, where the screening comprises activity or hybridization screening.

144. A method for generating a protein with an improved activity of interest, said method comprising:

a) selecting a wild-type DNA sequence from a library of DNA sequences isolated from a heterogeneous population of microorganisms;

b) introducing a mutation into the selected wild-type DNA sequence to form a mutated DNA sequence; and

c) determining whether a protein encoded by the mutated DNA sequence provides an improved activity of interest in comparison to a protein encoded by the wild-type DNA sequence.

145. The method of claim 144, wherein the library of DNA sequences is a library of cDNA sequences.

146. The method of claim 144, wherein the mutation is introduced by a method selected from error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis and site-specific mutagenesis.

147. The method of claim 144, comprising screening the library for an additional DNA sequence that provides the activity of interest, prior to introducing a mutation into the wild-type DNA sequence.

148. The method of claim 144, wherein the improved activity of interest comprises an improved enzymatic activity.

149. The method of claim 144, wherein the selection of the wild-type DNA sequence comprises contacting the wild-type sequence with a complementary nucleic acid.

150. The method of claim 149, wherein the complementary nucleic acid comprises a hybridization probe bound to a solid phase.

151. The method of claim 144, wherein the microorganisms comprise prokaryotes.

152. The method of claim 151, wherein the prokaryotes comprise microorganisms selected from Eubacteria and Archbacteria.

153. The method of claim 144, wherein the microorganisms comprise eukaryotes.

154. The method of claim 153, wherein the eukaryotes comprise microorganisms selected from fungi, algae and protozoa.

155. The method of claim 144, wherein the microorganisms comprise extremeophiles.

156. The method of claim 155, wherein the extremeophiles comprise organisms selected from thermophiles, hyperthermophiles, psychrophiles and psychrotropes.

157. A method for enhancing the activity of a protein encoded by a nucleotide sequence, said method comprising:

a) isolating at least two gene sequences encoding enzymes having a common characteristic from a library of gene sequences, wherein the library of gene sequences is derived from a heterogeneocls population of microorganisms;

b) mutating the gene sequences selected in (a); and

c) screening the mutated gene sequences to identify a gene sequence that encodes a protein having an enhanced activity of interest.

158. The method of claim 157, wherein mutating the gene sequences comprises a method selected from error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, *in vivo* mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis and site- specific mutagenesis.

159. The method of claim 157, wherein the library of gene sequences comprises cDNA sequences.

160. The method of claim 157, wherein the microorganisms comprise prokaryotes.

161. The method of claim 160, wherein the prokaryotes comprise microorganisms selected from Eubacteria and Archaebacteria.

162. The method of claim 157, wherein the microorganisms comprise eukaryotes.

163. The method of claim 162, wherein the eukaryotes comprise microorganisms selected from fungi, algae and protozoa.

164. The method of claim 157, wherein the microorganisms comprise extremeophiles.

165. The method of claim 164, wherein the extremeophiles are selected from thermophiles, hyperthermophiles, psychrophiles and psychrotropes.

166. The method of claim 157, wherein the improved activity is an enzymatic activity.

Please enter the following new claims:

C21 167. (New) The method of claim 133, wherein the operon produces a polyketide.

168. (New) The method of claim 133, wherein the operon produces an anti-cancer agent.

169. (New) The method of claim 133, wherein the operon produces an immunosuppressant.